PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF MALTO-OLIGOSACCHARIDES*

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SUMMARY

New preparative high-performance liquid chromatographic techniques that allow the practical isolation of milligram to gram quantities of malto-oligosaccharides from starch hydrolysates are described. High-resolution fractionations were achieved on preparative ($\approx$ 30 $\times$ 2.0 cm) columns packed with either (i) H^+-form cation-exchange resin, (ii) Ag^+-form cation-exchange resin, (iii) aminopropyl silica gel, or (iv) octadecyl (C_{18}) silica gel. Details are given for the preparation and packing of the first three phases and for the practical use of all four for rapid oligosaccharide fractionations. Although each column had some unique features, they all operated at surprisingly low flow-rates (2–12 ml/min) and pressures (15–1000 p.s.i.). In addition, most were relatively easy and inexpensive to assemble and operate. The use of these columns on standard analytical-scale chromatographs and also on automated preparative high-performance liquid chromatography systems, for the isolation of the title compounds, is described.

INTRODUCTION

Malto-oligosaccharides are linear α-1,4-linked D-gluco-oligosaccharides that are produced by the enzyme- or acid-catalyzed hydrolysis of amylose, the linear component of starch. Powders and syrups composed of mixtures of these and similar starch fragments are found in numerous sweeteners, confectionery items, food ingredients, and prepared foods. While these industrially prepared mixtures are readily available, purified samples of the individual malto-oligosaccharides are extremely scarce and in some cases, not obtainable. This is primarily due to the time consuming and difficult oligosaccharide fractionation techniques that are currently available, such as those utilizing column chromatography on cellulose[1], charcoal[2,3], or exclusion gels[4,5]. There is currently a great need for purified individual malto-oligosaccharides for use as experimental substrates for starch degrading enzymes, for exam-

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ination of their individual chemical, nutritional, and reported anti-microbial[6] properties, and as standards for various analytical techniques.

Considerable progress has been made in recent years in the analytical separation and quantitation of starch-derived oligosaccharides by high-performance liquid chromatography (HPLC) on columns packed with amine modified silica gel[7–9], reversed-phase silica gel[10,11], and cation-exchange resins[12–14]. A few attempts[15,16] have been made to apply these techniques to preparative carbohydrate isolations, but most researchers have been reluctant to adopt these methods because of their great expense and their requirements for special instrumentation. Therefore, in this report, we now describe several new practical methods for preparative HPLC of malto-oligosaccharides. Procedures are given for the preparation and packing of several different stationary phases, and for their use in preparative HPLC on standard analytical equipment as well as on automated preparative chromatographs. The advantages, disadvantages, and unique characteristics of each of these stationary phases are discussed. In many cases, preparative columns packed with these phases operate at low (< 5 ml/min) flow-rates and minimal (< 200 p.s.i.) pressures, are inexpensive to procure and operate, and allow rapid, high-resolution fractionation of gram quantities of malto-oligosaccharide mixtures.

EXPERIMENTAL*

Carbohydrates

Pure mono-, di-, and trisaccharides were purchased from Sigma. Samples of corn sirup solids, M-250 [with a dextrose equivalent (DE) of 25] and M-365 (DE = 36), were provided by Mr. J. Cannon of the Grain Processing Corporation. Prior to chromatography, these mixtures were deionized, as follows: 100 g of corn sirup solids were dissolved in 500 ml of water and then passed through consecutive glass columns containing 200 ml Amberlite IR-120 (H^+ form), 200 ml of Duolite A-561 (free base form), and finally 50 ml of Amberlite IRA-400 (carbonate form) resins. The sample and 1500 ml of column wash (water) were collected and evaporated under reduced pressure at < 35°C to yield 98.6 g of a white solid. High-molecular-weight [$\approx >$ degree of polymerization (DP) 8] oligosaccharides were also removed from this material by solvent partitioning. Hence, 20 g of the deionized sample were dissolved by stirring in 90 ml water for 30 min. Acetonitrile (110 ml) was then added with continued stirring and the sample was then quietly incubated (25°C, 8 h). The top layer (150 ml) of the resulting two-phase mixture was carefully decanted and evaporated to yield 4.2 g of a DP 1– *ca.* 7 oligosaccharide mixture. Samples to be injected into the Rainin Dynamax NH_2 column were also pretreated by passage of the deionized, solvent partitioned sample (10 g), dissolved in 50 ml of acetonitrile–water (35:65), through a glass column containing 50 g of non-HPLC grade aminopropyl silica gel (see below) equilibrated in acetonitrile–water (50:50). The sample, eluted rapidly with 200 ml of equilibrating solvent, was evaporated to dryness (9.1 g) as described. This off-line pre-column procedure was used to trap out sample components that bound

* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

very strongly to aminopropyl silica gel, and to thereby prevent subsequent damage to the preparative column.

Preparative stationary phases

Resins. AG 50W-X4 minus 400 mesh, and AG 50W-X8 minus 400 mesh (both H^+ form) were purchased from Bio-Rad Labs.; AG 50W-X4 20–30 μm size, Ag^+ form, was kindly provided by Mr. M. Gray, Bio-Rad Labs. Resins were partially ($\approx$75%) converted to the Ag^+ form and then treated to remove fines by the procedures of Scobell and Brobst[13].

Silica-based phases. Silica gel (irregular, 7 μm) was supplied by Rainin Instruments. Non-HPLC grade, acid-washed silica gels ($\approx$200–400 mesh) were obtained from Waters Assoc. Both types of silica gels were derivatized by reaction[17] with aminopropyltriethoxysilane (Sigma), to provide support loadings of approximately 7–8%. In a typical derivatization, 200 g of silica (previously equilibrated over saturated lithium chloride for 24 h) was stirred in 2 l hexane, as 50 g aminopropyltriethoxysilane was added. The mixture was stirred at 25°C for 20 min, and then filtered through a coarse glass filter. The white powder was immediately washed with 3–4 l of hexane and then dried (85°C) in a vacuum oven overnight. Support loadings were determined by measuring the weight lost after ashing. The HPLC grade aminopropyl silica gel so produced was custom packed into preparative Dynamax cartridges by Rainin Instruments. Dynamax preparative C_{18} bonded silica cartridges and preparative end fittings were purchased from Rainin Instruments.

Packing preparative columns

Empty, re-useable, stainless steel preparative HPLC columns, were purchased from Bio-Rad Labs. A preparative sized slurry packing reservoir, and a slurry packing apparatus (air driven liquid pump) was purchased from Alltech/Applied Science. Additional fittings for coupling the preparative reservoir to the pump and to the column, were purchased from Parker Hannifin. The column to be packed was mounted vertically, beneath the packing apparatus, and was filled with water with the bottom end fitting firmly attached and temporarily plugged. The open top of the column was attached, with appropriate fittings, to the bottom of the slurry packing reservoir. The reservoir was first filled with 150–200 ml of moist resin, that had been slurried in a minimum amount of water (total volume of 250 ml). Immediately, the top of the reservoir was attached to the packing pump by way of "Quick Connect" fittings. The plug was removed from the bottom end fitting of the column, and the packing pump, set to deliver 1000 p.s.i. using water as the packing solvent, was turned on. When the pressure of the system had reached this value (usually about 5–10 min), the pump was then stopped and the system pressure (and flow of solvent) was allowed to reach zero. The column was carefully removed from the reservoir, and an inlet end fitting and frit were quickly applied, before the pressurized resin could begin to extrude from the packed bed. If a slurry packing apparatus is not available the top of the slurry reservoir can be connected to a regular HPLC pump and solvent can be pumped through at a flow that will produce $\leqslant$1000 p.s.i. This technique results in less efficient, but useable columns.

Empty pre-columns (5 $\times$ 0.46 cm) were packed by attaching a 25-ml slurry packing reservoir, filled with a 50% slurry of AG 50W-X8 (H^+), to the inlet end of

the precolumn. The other end of the reservoir was attached to an HPLC pump, delivering water for 5 min at 6 ml/min. Inlet fittings were applied as previously described.

Preparative chromatography

Preparative HPLC was performed on a Gilson automated preparative gradient HPLC system, composed of two 303 pumps, an Apple IIe controller, a Rheodyne 7125 fixed-loop injector, a Kratos 520 column heater, and a Waters 403 preparative differential refractometer. For automated injections, a Gilson 302 pump, controlled by the microprocessor, was used to inject samples on an unattended basis. Fractions were collected either manually, or automatically, using the peak mode on a Gilson 202 fraction collector, interfaced to the microprocessor. All chromatograms were recorded on a Houston Instruments OmniScribe recorder. All solvents (HPLC grade) were continuously purged with helium during chromatography. Samples injected into resin or reversed-phase columns were dissolved in water. All samples injected into aminopropyl silica gel columns were dissolved in acetonitrile–water ($\approx$50:50). All samples were filtered through 0.2-μm Nylon 66 filters prior to injection.

As a precaution, all preparative columns were run with an in-line 2-μm filter (Rheodyne) placed between column and detector to prevent fines from the column from clogging the inlet line of the detector.

Samples collected from resin columns were passed through a mixed bed resin (such as Amberlite MB-3) to remove acidic eluents or traces of silver ion, prior to lyophilization. Samples collected from aminopropyl columns were evaporated at reduced pressures to remove acetonitrile, then treated with mixed-bed resins and lyophilized. These samples were re-dissolved in minimal water, filtered through 0.2-μm filters to remove silica gel, then re-lyophilized for storage.

After two sequential purifications on the aminopropyl column (see Results and discussion) the resulting malto-oligosaccharides had the following optical rotation values: [oligosaccharide, observed specific optical rotation (range of reported literature values)[18–20]] maltotriose, 157.7° (159–160°); maltotetrose, 170.5° (163–177°); maltopentaose, 178.0° (178–180°); maltohexaose, 168.4° (179–184°); maltoheptaose, 174.0° (179–186°).

RESULTS AND DISCUSSION

General principles of preparative HPLC of oligosaccharides

The goals of this study were to develop economical, preparative HPLC columns that would provide similar resolution, back-pressures and speed of separations to those given on analytical HPLC columns, while providing much larger (10–100 fold) sample capacity. To achieve these goals, we used wide-bore columns (2.0–2.5 cm I.D.) packed with HPLC-grade, and in some cases, non-HPLC grade stationary phases. In most cases described below, separations were first developed on a standard-sized analytical column, and then scaled up on the wider-bore preparative column packed with the same stationary phase. The appropriate flow-rate for the preparative column (F_p) was estimated by the following equation:

$$F_p = F_a[\text{I.D.}_p/\text{I.D.}_a]^2 \cdot L_p/L_a \quad (1)$$

where F_a is the analytical flow-rate, I.D.$_p$ and I.D.$_a$ refer to the inner diameters of the preparative and analytical columns, respectively, and L_p and L_a, refer to the respective column lengths. When this method was used to set preparative flow-rates, the retention times for each peak were nearly identical to those obtained on the analytical columns. In addition, the resulting column back-pressures were very low and the chromatograms were often indistinguishable from those from analytical separations, provided that moderate sample sizes were injected.

In these studies, high-resolution ($R \geqslant 1$, between adjacent peaks) separations were desired. Resolution values were estimated by the following equation:

$$R = \Delta t / t_w \tag{2}$$

where Δt is the distance between adjacent peaks, and t_w is the average width of the peaks at the baseline. When two similar-sized peaks were separated by $R \geqslant 1$, each peak was isolated with less than 5% overlap, providing very pure fractions. In difficult separations, resolution values $\geqslant 1$ were not always possible, and fractions resulting from those separations were predictably less pure. Although mathematical relationships between sample loading capacity and column dimensions were not determined here, we noted that the resolution between peaks was inversely proportional to the amount of sample loaded. Thus, for each of the columns used, we empirically determined the optimum sample size that would permit the greatest throughput of high purity oligosaccharides.

Preparative HPLC on strong cation-exchange resins

Cation-exchange resins are routinely used for the analytical HPLC separation of malto-oligosaccharides[12–14]. On these resins, oligosaccharides are separated on the basis of size-exclusion and ligand-exchange mechanisms[21,22] and both the internal pore size of the resin and the presence of bound metal ligands, affect the quality of the separation. Most analytical HPLC columns are packed with either 4% or 8% crosslinked resins. Because the former types have larger pore sizes and they resolve larger oligosaccharides, they were used exclusively for these preparative applications. The effect of resin ionic form (ligand) on oligosaccharide separation can be clearly seen in Fig. 1. In Fig. 1a, a malto-oligosaccharide mixture is separated on a preparative column packed with a non-HPLC-grade, 4% crosslinked resin in the H^+ form. Partial separation of oligosaccharides from DP 1 to 5 is accomplished in less than 25 min. A similar column packed with the same resin, but in the Ag^+ form (Fig. 1b), separated oligosaccharides up to at least DP 6, under the same chromatographic conditions. The advantages of the latter column are obvious: greater selectivity (resolving capability) and a simpler mobile phase. By decreasing the flow-rate through this column to 1 ml/min (Fig. 2a), malto-oligosaccharides up to DP 8–9 were partially resolved.

The effect of stationary phase particle size (and monodispersity) on chromatographic resolution can be seen by comparison of Fig. 2a and b. The resin in Fig. 2b, AG 50W X4 (20–30 μm), has a small average particle size (25 μm) and a narrow size distribution ($\pm$ 5 μm). This column resolved malto-oligosaccharides up to DP 11. The resin used in Fig. 2a was identical to that in Fig. 2b, except for particle size. The non-HPLC-grade resin, designated as minus 400 mesh size, has a very broad size

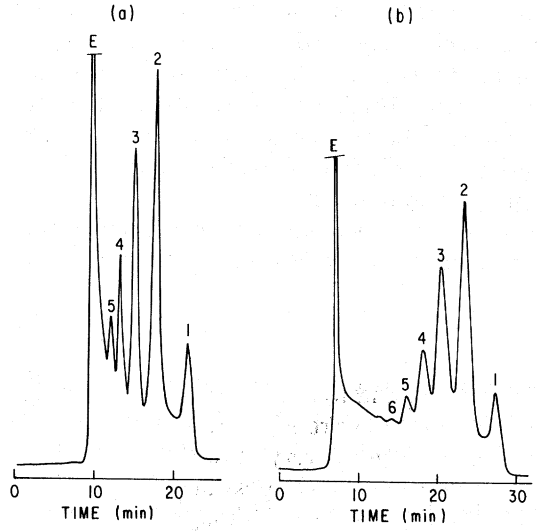

Fig. 1. Separation of oligosaccharides in a deionized commercial corn sirup solids sample (M-365) on preparative (30 × 2.0 cm) HPLC columns packed with cation-exchange resins (AG 50W-X4 minus 400 mesh) in the H^+ or Ag^+ form. (a) H^+ form resin eluted with 0.01 *N* sulfuric acid at 2.0 ml/min and ≈ 200 p.s.i. (b) Ag^+ form resin eluted with water at 2 ml/min and ≈200 p.s.i. Other conditions: column temperature, 85°C, sample size, 5 mg in 50 μl; detection, refractive index at 16×. Numerals above peaks refer to DP values; peak marked "E" contains excluded material.

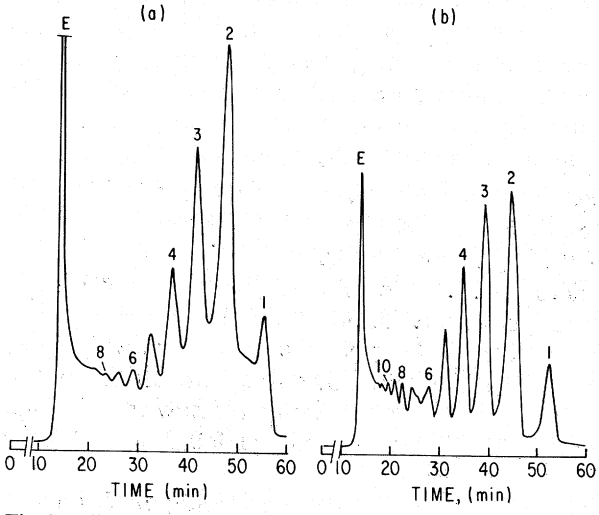

Fig. 2. Effect of resin particle size on preparative HPLC separation of malto-oligosaccharides. (a) Column packed with AG 50W-X4 (Ag^+ form) minus 400 mesh size, eluted at 85°C with water at 1 ml/min and 180 p.s.i. Sample was deionized M-365 corn sirup solids (5 mg in 50 μl). Refractive index detection at 8×. (b) Column packed with AG 50W-X4 (Ag^+ form) 20–30 μm size, eluted at 85°C with water at 1 ml/min and 100 p.s.i. Same sample as in (a) except 100 mg (in 250 μl) was injected. Refractive index detection at 128×. See Fig. 1 for explanation of peak designations.

distribution and larger average size (≈ 50 μm). Although the selectivities of the two resins were identical, the increased efficiency of the smaller resin particles led to an obvious increase in overall column resolution, even when much larger loads were injected onto the column (see legend Fig. 2). Other factors which effected malto-oligosaccharide separations on resin columns were packing efficiency and column temperature. All columns evaluated here were packed at high pressures (1000 p.s.i., see Experimental section) and efforts to pack such columns by gravity sedimentation led to less acceptable results, especially for the non-HPLC-grade resins. In addition, all cation-exchange resin columns were run at 85°C since use of lower temperatures gave considerably poorer resolution.

The separations shown in Figs. 1 and 2 are similar to those on analytical columns packed with the same phases. Contrary to previous reports[2,3] which suggested that 4% resins could not be used in wide-bore columns because of high back-pressures and subsequent compression of the resin, these columns consistently ran at nominal back-pressures (10–250 p.s.i.). The low operating flow-rates of these columns also allowed them to be used on conventional HPLC systems, without any modifications other than the installation of larger injector loops.

The loading capacity of each of the columns in Fig. 2 was examined, by injecting increasing levels of malto-oligosaccharide samples and calculating the resulting resolution between adjacent peaks. Because only oligosaccharides with DP values between 3 and 7 were of interest in this study, the higher oligosaccharides and dextrins were removed from the sample prior to chromatography by solvent fractionation (see Experimental). Representative chromatograms, resulting from optimum sample loads on each column are shown in Fig. 3. Identical 250-μl (125 mg) samples were injected onto the columns packed with non-HPLC-grade (Fig. 3a) and HPLC-grade (Fig. 3b) resins. Calculated resolution values are indicated between adjacent peaks.

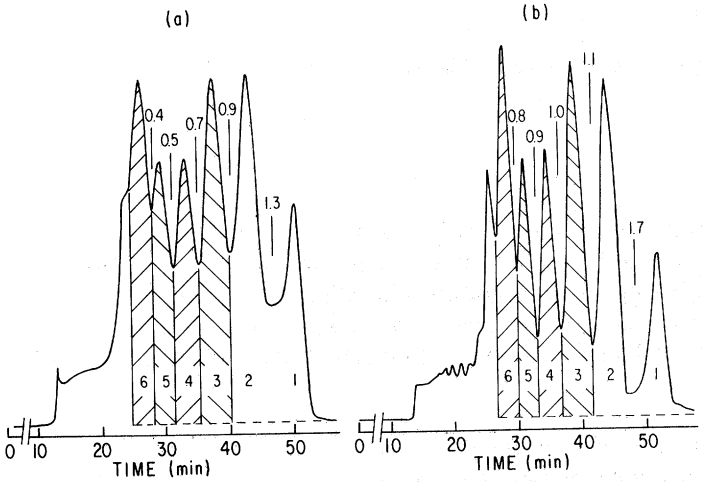

Fig. 3. Isolation of malto-oligosaccharides by preparative HPLC on HPLC- and non-HPLC-grade resin-packed columns. (a) Column packed with AG 50W X4 (Ag^+ form) minus 400 mesh size. (b) Column packed with same resin as in (a) except 20–30 μm size. Other conditions as follows. Column dimensions, 30 × 2.0 cm; temperature, 85°C, mobile phase, water at 1.1 ml/min and ≈ 150 p.s.i.; samples, 125 mg (250 μl) of deionized, solvent partitioned M-250 corn sirup solids. Refractive index detection at 128×. Numerals inside peak areas refer to DP values. Numerals between peaks are calculated resolutions values.

Under these conditions of loading, neither column separated all oligosaccharides with R values $\geqslant 1$, but both columns allowed a substantial fractionation of these mixtures. The actual weights and purities of these isolated fractions are given in Table I. Approximately equal amounts of each oligosaccharide were isolated on each column in the same amount of time. The purity of oligosaccharides from the non-HPLC grade resin column averaged about 72% while those from the HPLC grade resin column were considerably purer ($\approx$90%). Although the latter column is clearly superior in its ability to produce pure fractions, this fact alone does not necessarily justify its use. The non-HPLC resin was obtained for a nominal cost, while the expense of the HPLC grade resin was almost two orders of magnitude higher. In both cases, fractions had to be purified by one more pass through the column to reach 95–99% purity.

TABLE I

COMPARISON OF THREE STATIONARY PHASES FOR PREPARATIVE HPLC OF MALTO-OLIGOSACCHARIDES

Phase	*Sample size*	*Run time (min)*	*Isolated malto-oligosaccharides per run (mg) (% purity)*				
			DP 3	*DP 4*	*DP 5*	*DP 6*	*DP 7*
AG 50W-X4 Ag^+ form							
−400 mesh*	125 mg (250 µl)	53	19(71)	15(63)	13(66)	21(89)	–
20–30 µm**	125 mg (250 µl)	55	23(88)	13(88)	11(88)	23(92)	–
Dynamax NH_2***	500 mg (2.0 ml)	32	80(98)	48(95)	52(94)	100(96)	46(81)

* For column dimensions and conditions see Fig. 3a.
** See Fig. 3b.
*** See Fig. 4b.

Regardless of the resin used, these columns were extremely durable and operated for over a year without loss of resolution when samples were carefully deionized, prior to injection. By repetitive, overlapped injections, spaced 30 min apart, up to 2 g of malto-oligosaccharide mixture could be processed in an 8-h period, resulting in the isolation of 150 to 350 mg of each of the four (DP 3-6) oligosaccharides shown in Fig. 3.

Preparative HPLC on C_{18} bonded silica gel

A commercially available, preparative sized (25 × 2.14 cm) C_{18} bonded silica gel column was used for malto-oligosaccharide fractionation (Fig. 4a). Oligosaccharides are eluted from these columns in an opposite order to that seen on resin based columns; lowest-molecular-weight sugars are eluted first. Although the column operated at low flow-rates and back-pressures and used a simple mobile phase (water), the run times were long, and each oligosaccharide larger than maltose gave a broad,

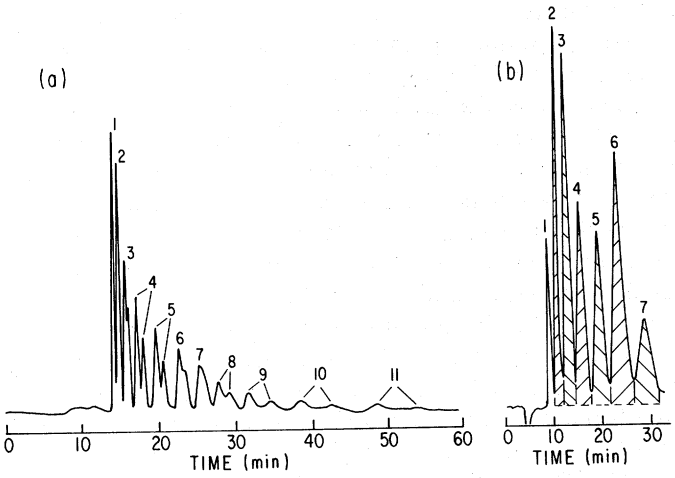

Fig. 4. Preparative HPLC of malto-oligosaccharides on reversed-phase (a) and normal-phase (b) columns. (a) Dynamax C_{18} preparative column (25 × 2.14 cm) eluted with water at 3 ml/min and 100 p.s.i. at room temperature. Sample size: 12 mg (in 100 μl) of same sample shown in Fig. 3. Refractive index detection at 16×. (b) Column dimensions same as (a) but packed with aminopropyl silica (APS) gel, eluted with acetonitrile–water (55:45) at 12 ml/min and 1000 p.s.i., at room temperature. Sample size was 500 mg (in 2 ml) of same sample used in Fig. 3 except in b, where sample was pre-treated by passage through a column of non-HPLC-grade APS gel, as described in Experimental. Preparative refractive index detector at 64×. Numerals above peaks refer to DP values.

double peak, due to separation of the α- and β-anomeric forms of each oligosaccharide. This observation, which has been reported[10,11] for analytical reversed-phase HPLC separations, caused peaks to overlap when samples larger than 12–15 mg were injected. Hence, the capacity of the column was surprisingly low and was only useful for isolating mg quantities of the oligosaccharides. This column must occasionally be washed with methanol to restore its capacity, but otherwise, was exceptionally durable in this aplication.

Preparative HPLC on aminopropyl silica gel

Carbohydrates are separated on aminopropyl silica (APS) gel columns on the basis of normal-phase partitioning mechanisms[24] and oligosaccharides are eluted from them in order of increasing molecular weight, as on C_{18} columns. APS columns were not as convenient to use as cation exchange types since they required higher flow rates and binary mobile phases (acetonitrile–water mixtures). However, their sample capacities were significantly larger. Fig. 4b represents the separation of 500 mg of a malto-oligosaccharide mixture on a custom-packed preparative APS column (Dynamax NH_2). This preparative chromatogram was similar to analytical-scale separations and the calculated resolution between peaks was in every case, greater than 1. Although larger amounts (up to 750 mg) of sample were injected with similar results, this practice is not recommended since repeated injections of that level caused noticable losses in column efficiency. The weights and purities of fractions isolated from Fig. 4b are given in Table I. The amounts isolated are four- to five-times more than those isolated per injection on the cation exchange columns and the purities of

maltotriose through maltohexaose averaged about 96%. Chromatographic runs required only about 30 min, and unlike the separations on the cation exchange resin columns, maltoheptaose was also isolated in relatively high purity.

The Dynamax NH_2 column was also used on a commercially-available automated preparative HPLC. On this system, a microprocessor was programmed to control flow-rates, to inject samples, and to wash strongly-retained sample components from the column with pure water between injections. Oligosaccharides were collected automatically by a peak-sensing fraction collector, allowing the entire process to be carried out in an unattended manner. A series of twelve sequential 500-mg injections was carried out on this system and two of these are shown in Fig. 5a. Analysis of the isolated fractions (Fig. 5b) revealed purities indistinguishable from those collected manually. The amounts of each oligosaccharide isolated during this 10-h period were: maltotriose: 0.96 g; maltotetraose: 0.58 g; maltopentaose: 0.62 g; maltohexaose: 1.20 g; maltoheptaose: 0.55 g. Each of these fractions were rapidly purified on the column once more and the resulting malto-oligosaccharides were judged to be pure by HPLC (>99% based on HPLC peak area, except 97% for DP 7) and optical rotation measurements (see Experimental). Although some commercial corn sirup solids mixtures may contain branched oligosaccharides (containing α-1,6 linkages in addition to α-1,4 types), we found no detectable levels in the sample of M-250 fractionated here. Isomaltose and isomaltotriose are known to separate well from maltose and maltotriose on APS columns, and no peaks for these or the larger branched saccharides were found. In addition, when these oligosaccharides were examined by high-resolution (300 MHz) ^1H NMR spectroscopy[25], the spectra for each oligosaccharide was identical to that of pure, standard malto-oligosaccharides. No resonances from α-1,6-type linkages were observed for even the larger (DP 5–7) oligosaccharides.

The Dynamax NH_2 column was capable of providing greater sample throughput and providing purer fractions than any of the other columns tested. However, it also had certain limitations. First, it required flow rates that were higher than many analytical HPLC systems were capable of delivering, and that were above the rec-

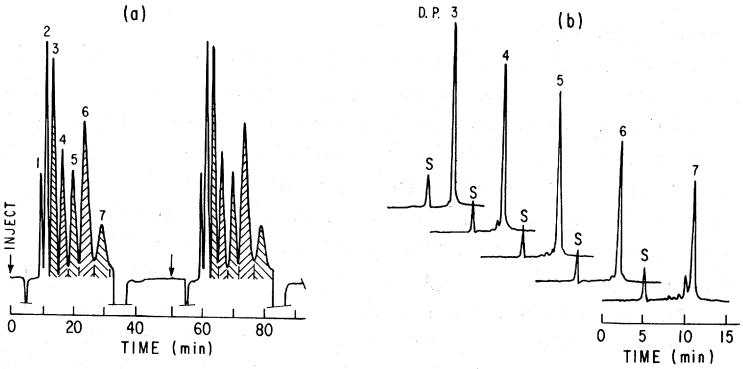

Fig. 5. Automated preparative HPLC of malto-oligosaccharides. (a) Two automated injections of 500 mg of pre-treated oligosaccharide mixture, as described in Fig. 4b. Injection points are marked by arrows. (b) Analytical HPLC assays of fractions automatically collected in (a). Column, IBM amino (25 × 0.46 cm) eluted with acetonitrile–water (75:25) at 1 ml/min. Numerals refer to DP values. S refers to solvent peaks.

ommended maximum value for analytical-type refractive index detectors. Secondly, APS phases are known[26] to be somethat unstable and prone to gradual loss of capacity. APS phases also gradually dissolve in the mobile phase and leave a void in the column inlet. This leads to tailing of peaks, and decreased peak resolution. The Dynamax column, however, has a threaded inlet end-fitting, that can be tightened down when necessary, to eliminate voids in the bed. We found that this process worked well for about three months of use, until no more compression of the fitting was possible. At this point the column was no longer usable. During this three month period, however, there was no measurable loss of capacity of the stationary phase, only a loss of resolution caused by column voids. Because of the tendency of the stationary phase to dissolve, it was necessary to remove silica residue from collected samples by filtration as outlined in the Experimental section. This gradual deterioration can be somewhat delayed by installing silica saturator columns[26] in the mobile phase inlet lines, and by adding phosphate buffers[26] to the mobile phase. The latter, though, is not convenient for preparative purposes, since these buffers must be removed from the final fractions. To avoid deactivating the column by sample contaminants and high molecular weight carbohydrates, we pre-purified all samples by treatment with APS gel in a low pressure open column. Preparation of the phase was easy and inexpensive and was more practical than using small precolumns of APS in-line with the preparative column.

CONCLUSIONS

The development and practical use of several types of preparative HPLC columns for fractionation of malto-oligosaccharides have been described. Preparative HPLC on wide-bore columns resulted in excellent separations at low flow-rates and back pressures. For small scale ($\leqslant$ 1 g) applications, durable resin-based columns were used inexpensively on conventional HPLC systems. The resulting fractions were relatively concentrated and contained no additional column- or mobile phase-derived contaminants. Reversed-phase (C_{18}) silica gel columns had very low sample capacity, but provided mg-level samples suitable for spectroscopic studies. APS columns had the greatest capacity and highest resolving capability of all the phases tested and allowed the multi-gram fractionation of malto-oligosaccharides. They were also, however, the least durable of the columns tested, and they required careful pretreatment of samples to avoid rapid loss of capacity. The application of these columns to the separation of water soluble oligosaccharides from chitin, cellulose, and other carbohydrate polymers, will be reported elsewhere.

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